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Nuclein Synthesis in the Animal Body

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Nuclein Synthesis in the Animal Body

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Modern investigations have led physiologists to the belief that in the normal processes of metabolism, the proteins of the tissues of the animal are the products of a regeneration of these bodies from comparatively simple cleavage products of those proteins taken as food.[1]

Our knowledge of the fate of nucleins in the body, and of the origin of the body nucleo-proteins, is less clear than of the simple proteins. The nucleo-proteins are complexes, consisting of simple proteins in union with nucleic acid, the latter containing a high content of phosphorus in combination with purine and pyrimidine bases and a carbohydrate group. The earlier investigators established the fact that the proteoloytic digestive enzymes, pepsin and trypsin, do not attack nucleic acids in such a manner that purine bases are liberated.[2] More recently Abderhalden and Schittenhelm[3] have studied the behavior of thymus nucleic acid with pancreatic juice of the dog, and found that this substance is changed in some manner so that the characteristics of nucleic acid are lost, but without the liberation of purine bases. They likewise found that thymus nucleic acid, when digested with extracts of the pancreas and intestinal mucosa of the cow, was speedily liquefied and purine bases set free. This the authors attribute to the presence of intracellular enzymes in such extracts.

The behavior of nucleo-proteins and their cleavage products, the nucleins and purine bases, with individual organs and tissue

[1] Hugo Lüthje, Ergebnisse der Physiologie, 1908, 7: 795. A résumé of the researches bearing on this subject.
[2] Iwanoff, Ztschr. Physiol. Chem., 1903, 39: 31. References to the older literature.
[3] Abderhalden and Schittenhelm, Ztschr. Physiol. Chem., 1906, 47: 452.

extracts, has received much attention during the last few years.[4] The existence of four distinct classes of enzymes concerned with the transformations of purines in the body seems to be well established:[5]

1. Nucleases; which liberate purine bases from the nucleic acid molecule.

2. Deamidizing enzymes; which liberate ammonia from adenine and guanine, forming oxy-purines, hypoxanthine and xanthine.

3. Oxidizing enzymes; which oxidize hypoxanthine and xanthine to uric acid.

4. Uricolytic enzymes; which destroy uric acid.

Almost every organ and tissue of the animal body seems to be endowed with the power to bring about one or more changes in the nucleic acids or their products, all of which lead to the final destruction of the component parts of the molecule. These facts lead to the question: What kind of phosphorus compounds can the animal utilize for the elaboration of the phosphorus containing complexes of its cell nuclei? and, Is an exogenous supply of purine bases essential to nuclein formation?

The work of the investigators cited above all points to a destruction of the nucleic acids taken with the food, rather than a direct transposition of nucleic acid complexes of exogenous origin, and a substitution of these for the portions of the nuclei of the living cells broken down during metabolic activity.

Steinitz[6] attempted to throw light on the question as to whether the formation of nucleo-proteins is a chain of syntheses involving inorganic phosphoric acid, by studying the nitrogen and phosphorous retention in a dog fed: (a) a phosphorus free protein (myosin) combined with carbohydrate fat and inorganic salts, the phosphorus being supplied in inorganic form, and (b) a phosphorized protein (vitellin) combined with the same substances but without the phosphates. He found a better retention of phosphorus, but poorer retention of nitrogen when vitellin was given and a better retention of nitrogen, but insignificant storage of phosphorus when myosin supplied the protein of the ration. His experiments were conducted only five to eight days. Leip-

[4] Bloch, Biochem. Centbl., 1906, **5:** 521, 561, 817, 873, gives an extensive résumé of the literature.

[5] Mendel and Mitchell, Amer. Jour. Physiol., 1907, **20:** 97.

[6] Steinitz, Arch. Physiol., [Pflüger], 1898, **72:** 75.

ziger[7] using the same dog, repeated Steinitz' experiment using edestin as the phosphorus free protein and confirmed his observations in a 7-day trial. Zadik[8] and Ehrlich[9] employing edestin and casein reached the same conclusion as a result of similar experiments.

The above experiments were all of short duration and can hardly be looked upon as positive proof of the point in question. It is well known that the elimination of phosphorus is not necessarily constant during a brief period and is influenced by numerous factors, especially by the relative amounts of the alkaline earth and alkali salts in the food.[10]

In an experiment by Hart, McCollum and Fuller[11] it has been shown that when pigs were fed on a ration containing a very low phosphorus content, and which proved inadequate for the maintenance of the animals, the addition of phosphorus in the form of calcium phosphate, corrected all of the pathological disturbances and led to normal growth and development. Their experiment did not furnish proof of a nuclein or phosphatide synthesis from inorganic phosphates, since their ration still contained small amounts of phosphorus in unknown forms. It was not found possible to secure a basal ration entirely free from phosphorus, in sufficient quantity for work with large animals.

It was the purpose of the writer in undertaking the present series of experiments, to demonstrate whether an animal can rely wholly upon inorganic phosphorus forms for its supply of this element.

Such a series of experiments involves the maintenance of animals during the growing period upon a ration of artificially prepared foodstuffs, rendered phosphorus free by appropriate methods of purification, inorganic forms of phosphorus being added. This is necessary since none of our naturally occurring protein containing foodstuffs are free from organic forms of phosphorus.

Several attempts by other investigators to maintain animals on a ration made up of relatively pure proteins, carbohydrates, fats and inorganic salts, have been wholly or partially unsuccessful.

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7 Leipziger, Arch. Physiol., [Pflüger], 1899, **78:** 402.

8 Zadik, Arch. Physiol., [Pflüger], 1899, **77:** 1.

9 Ehrlich, Stoffwechselversuche mit P-haltigen und P-freien Eiweisskörpern. Inoug-Diss. Breslau. 1900.

10 Ehrström, Skand. Arch. Physiol. 1903, **14:** 82–111.

11 Hart, McCollum and Fuller, Amer. Jour. Physiol. 1909, **23:** 246. Wis. Agr. Expt. Sta. Research Bul. 1.

Socin[12] and Hall[13] attempted to maintain mice on a ration consisting of casein, fat and sugar or starch and inorganic salts. Socin gave in one experiment also hemaglobin to furnish a supply of organic iron. His mice lived no longer than 33 days in any case.

Hall added cellulose to his ration to serve as an irritant to the digestive tract and carniferrin to supply organic iron. In no case did he succeed in keeping the animals alive on such a ration more than 40 days.

Falta and Nöggerath[14] fed white rats on rations in which the protein was supplied by relatively pure proteins of different sources. Carbohydrates, fats and inorganic salts were given in addition. With a ration in which the nitrogen was given as serum albumin and casein their animals died after 51 to 53 days, having lost weight from the beginning of the experiment. When the nitrogen was given as ovalbumin the rats lived 83 to 94 days. In a trial in which serum albumin, ovalbumin and casein were given together in addition to carbohydrates, fats and inorganic salts the rats lost weight as in the preceding experiment and died in from 71 to 94 days. The addition of sodium nucleinate, cholesterin and lecithin gave no better results. The authors believed that the steady decline of their animals was due to either insufficient intake or to lack of utilization of the food consumed rather than an actual insufficiency in the ration.

L. Jacob[15] has contributed some very instructive experiments in this field. Doves were fed a ration consisting of casein, starch and sugar, fat and inorganic salts in the form of ash of milk. The ration was calculated to supply protein, carbohydrate, fat and ash in the same proportions found in the wheat grain. The mixture was pressed into pellets of uniform size. The doves in two experiments were dead or in a dying condition at the end of 17 days. When meat powder was substituted for casein the results were the same. On sectioning, the crops were seen to be filled with a compact dough-like mass. The writer attributed the outcome of the experiment to this difficulty. The birds could not expel the food from the crop, and thereafter vomited everything they attempted to eat. Death was therefore due to inanition.

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12 Socin, Ztschr. Physiol. Chem., 1891, 15: 93.
13 Hall, Arch. Anat. u. Physiol. 1896, p. 49.
14 Falta and Nöggerath, Beitr. Chem. Physiol. Path., 1905, 7: 313.
15 Jacob, L., Ztschr. Biol., 1906, 48: 19.

In one experiment in which cellulose was added better results were obtained. Jacob calculated the food value of the pellets fed to the dove and observed that during the four weeks of the experiment the total intake was equivalent to only 70 per cent of the energy requirement of the bird. The loss in weight was proportional to the energy deficit of the food taken.

The same investigator experimented with rats, feeding a mixture of casein, carbohydrate and fat, to which inorganic salts were added. The ration was mixed with cellulose. The rats lost weight steadily from the beginning and died after 42, 73, and 124 days respectively, after the loss of about 40 per cent of their body weight. The difference in the ability of individuals to withstand such a diet is strikingly apparent.

Zadik[16] prepared four different rations as follows:

1. Casein, nutrose, bacon, starch, meat extracts and meat salts.
2. Nutrose, bacon, starch and salts.
3. Casein, bacon, starch and salts.
4. Vitellin, bacon, starch and salts.

Alternating these rations he was able to mainain a dog 36 days during which time the animal gained 200 grams in weight. At this point the dog became ill and diarrhoea and vomiting necessitated the cessation of the experiment. The dog was unable to eat meat without vomiting. Zadik believed the interruption of the experiment resulted from catarrh of the bladder brought on by the continued use of a catheter.

G. Marcuse[17] fed a dog casein, rice starch, bacon and salts during 11 days and observed a gain of 730 grams in the animal's weight, and a retention of 11.25 per cent. of the nitrogen ingested. The dog likewise retained phosphorous.

Henriques and Hansen[18] fed white rats with a mixture of casein, fat, sugar, cellulose and salts during 13–17 days and observed that they gained in weight and retained nitrogen.

Willcock and Hopkins[19] have reported that a diet containing only zein as a nitrogenous constituent is unable to maintain growth in young mice. The addition of tryptophane, an amino which acid is absent from zein, while not making it capable of maintaining growth, promotes the wellbeing of the animals and greatly prolongs the survival period.

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16 Zadik, Arch. Physiol. [Pflüger], 1899, 77: 1.
17 Marcuse, G., Arch. Physiol. [Pflüger], 1896, 64: 223.
18 Henriques and Hansen, Ztschr. Physiol. Chem., 1904–5, 43: 417.
19 Willcock and Hopkins, Jour. of Physiol., 1907, 35: 88–102.

Another class of experiments should be mentioned in this connection. Abderhalden and Rona[20] were unable to keep a dog in nitrogen equilibrium with a pancreatic digest of casein. Lesser[21] could not obtain nitrogen equilibrium in a dog fed with a pancreatic digest of fibrin.

Plosz[22] attempted to substitute peptone for protein. He fed a young pup for 18 days on a mixture of peptone, sugar and fat and a salt mixture. The dog gained 501 grams or 37.5 per cent of its body weight.

Loewi[23] fed a dog with a mixture of ammoacids and other simple products resulting from the auto-digestion of pancreas. The mixture did not give the biuret reaction. During 11 days the dog gained 0.72 grams of nitrogen and 0.649 gram phosphorus. Loewi concluded that with these simple substances the animal can replace body tissue.

In the experiments just described the constituents of the rations used were relatively pure chemical substances except in Zadik's employment of meat extracts and bacon. The former contains unknown nitrogen and phosphorus compounds and the latter connective tissue and some cellular materials.

We have now to mention several experiments in which a certain amount of naturally occurring mixtures were added to rations composed mainly of simple chemical substances of known composition.

Salkowski[24] fed a ration of eucasein (NH_3 caseinate), bacon. rice and meat extract. A dog ate this for 10 days and increased 285 grams in weight. At the end of the period it refused to take food. In another experiment with the same mixture in different proportions a second dog ate the ration for a period of 24 days, gaining 285 grams in weight. It retained nitrogen and was in a normal condition.

Röhmann[25] fed mice on a mixture of casein, egg albumen, vitellin, nucleoprotein from liver, wheat starch, potato starch, margarine and ether extract of egg yolk. To these he added

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20 Abderhalden and Rona, Ztschr. Physiol. Chem., 1905, 44: 198.
21 Lesser, Ztschr. Biol., 1904, 45: 497.
22 Plosz, Arch. Physiol. [Pflüger], 1874, 9: 323, cited from Maly's Jahresbericht der Tier-Chemie, 1875, 4: 21.
23 Loewi, Arch. Expt. Path. u. Pharmakol., 1902, 48: 303–330. Cited from Maly's Jahresber. Tier-Chemie, 1903, 32: 684.
24 Salkowski. Deut. Med. Wochnschr. 1896, No. 15, p. 225.
25 Röhmann, Klin. Ther. Wohnschr., 1902, 40: 1. Cited from Maly's Jahresber. 1904, 33: 823.

sodium and magnesium citrates, calcium lactate, calcium phosphate, dipotassium phosphate and sodium chloride.

The mice lived 96 days on this ration, produced young and the young continued 94 days on the same ration to which was added about 4 per cent malt. They in turn produced young.

Weiske[26] studying the effect of a calcium—poor ration on the composition of the bones, fed a goat (6–7 years old) on wheat straw which had been thoroughly extracted with hydrochloric acid. To this he added casein, sugar, starch and sodium chloride.

A second animal received extracted straw, casein, sugar, starch, sodium chloride and sodium phosphate. A third received the same ration as did the second except that calcium carbonate was substituted for sodium phosphate.

The second goat would not eat the ration. The third ate the ration readily during 42 days when it began to leave some of its feed. There were no signs of illness except that it grew languid toward the end of the experiment.

In another experiment in which sodium phospnate was used instead of calcium carbonate the animal showed no signs of illness but grew feebler day by day and died on the 50th day.

In a third series of experiments Weiske and Wildt[27] fed 2½ months old lambs with the same mixture to which calcium carbonate and sodium phosphate were added. The experiment was continued 55 days.

No. 1 decreased in weight from 46 to 32 pounds.

No. 2 decreased in weight from 47 to 34 pounds.

E. Voit calculated from the data furnished by Weiske and Wildt that the intake of food by the animals in these experiments was too small for maintenance, and attributed their loss of weight to inanition.

When we consider the causes leading to the unsatisfactory results in the experiments above described, several possibilities are suggested:

1. The animals may have failed because of a lack, wholly or in part, of certain organic complexes in the food given, which the body was not able to supply through its synthetic power from the materials at hand.

2. Certain of the ash constituents essential to the life of the animal can be utilized only when presented in certain organic

[26] Weiske, Ztschr. Biol., 1872, **7**: 1 Abhg. p. 179; 2 Abhg. p. 333.
[27] Ibid. 1873, **8**: 239. Wildt, ibid. **8**: 266.

combinations, whereas in several of the experiments described they were given as inorganic salts. Iron and phosphorus might be especially mentioned in this connection.

3. The physical character of the food, especially in respect to lack of bulk and irritating power in the digestive tract.

4. The sameness of the ration.

5. The psychical factor of palatability as influencing the intake and utilization of food.

Concerning the two first-mentioned factors, it would seem that they have been excluded by the experiments described. Falta and Nöggerath met this objection by feeding sodium nuclinate, lecithins and a variety of proteins with no better results than have been obtained by others with much simpler mixtures in which the iron and phosphorus were given in inorganic forms.

That the physical character of the food might be responsible for the observed inadequacy of the rations in certain cases must be admitted. Lunin,[28] however, found that mice lived indefinitely, without any ill effects, on evaporated milk alone. Socin[29] fed mice on egg yolk, starch and cellulose during 99 days and they remained in excellent condition.

The writer in a preliminary experiment designed to show whether the cellulose was an important factor in Socin's experiment, fed five half-grown mice on boiled egg yolk alone from May 19 to October 7. The five mice together at the beginning weighed 37 grams. At the end of the experiment they weighed 16, 17, 20, 14, and 14 grams respectively, their collective weight being 81 grams. This ration contained no indigestible matter but was quite sufficient for normal growth and development of the mice.

In another experiment two young white rats were fed on egg yolk alone. The eggs were hard boiled and the whites completely separated. No. 13 (male) gained weight from 71 grams at the beginning to 167 grams at the end of 18 weeks. No. 14 (female) gained weight from 40 grams to 160 grams during the same period. Just before the close of the experiment she gave birth to eight young which she had made entirely from egg yolk. The ration in addition to being entirely free from indigestible matter contained no carbohydrate. The idea that the sameness of the

28 Lunin. Ztschr. Physiol. Chem., 1881, 5: 31.
29 Socin, Ibid., 1891, 15: 93.

ration necessarily leads to a failure of the appetite is not compatible with these experiences.

The work of Pawlow[30] and his students seems to furnish a more satisfactory explanation of the question. The psychic influence of palatability is one of the most important factors in nutrition, either human or animal. Pawlow and his students have shown that the character of the secretions of the digestive glands is profoundly influenced, both in quantity and quality, by the mental sensations accompanying the taking of food. With food possessing little taste, and giving little or no pleasurable sensations on eating, the digestive secretions are scanty and of low digesting power, while the mere enjoyment of eating very palatable foods, even when these never actually enter the stomach, as was the case in his system of "sham feeding," the gastric and pancreatic juices produced by dogs are very abundant and of high digesting power.

Lusk[31] says "Not only the quantity of the food makes for the wellbeing, but the quality as well. No amount of actual fuel value could compel the American soldiers of the Spanish war to eat the "embalmed beef" furnished by the government. The flavor is to the man what oil is to the battleship. Without flavor in the food the digestive apparatus does not run smoothly."

PURPOSE OF THESE EXPERIMENTS

In the experiments to be described, the hope of success was based mainly on the belief that a ration composed of pure proteins, carbohydrates, fats and the necessary salts, could be made sufficiently palatable to insure a satisfactory intake and utilization of food. The primary object was to limit the phosphorus supply wholly to organic forms. All conceivable devices compatible with this end were resorted to in order to change the taste and relieve the monotony of food supplied from day to day.

The first experiment was carried out with two lots of three rats each. Instead of full grown animals, white rats about half grown or somewhat under half the adult weight were employed since it was desirable to obtain an increase in body weight. It was believed also that at this actively growing period there is a better ability to utilize food than when growth has ceased.

30 Pawlow, Work of the Digestive Glands, 1902, p. 76.
31 Lusk, Science of Nutrition, 1906, p. 189.

PREPARATION OF THE FOOD MATERIALS

The proteins of the food of Lot I consisted of edestin and zein. It was found impossible to prepare any other proteins in the necessary quantity in a phosphorus free condition. Even with these easily obtainable proteins the necessary degree of purity was attained only with much persistence, the proteins retaining a trace of phosphorus with surprising tenacity. Zein was purified by pouring its alcoholic solution into water, and repeating this a large number of times. It was then dried, ground as finely as possible and soaked in 0.5 per cent HCl. This treatment was very effectual in removing phosphorus. When the extraction was nearly complete the protein was dissolved in alcohol, reprecipitated by pouring into water and the process of drying and extracting with HCl repeated. By presenting new surface for extraction the phosphorus removed by soaking with HCl was greatly increased.

Edestin was repeatedly crystallized by cooling its dilute salt solutions. The content of phosphorus steadily but slowly decreases during this process of purification.

The standard of purity for all foods was the failure of the qualitative test for phosphorus by the Neumann method on 5 grams of material. Glucose was not at hand in this degree of freedom from phosphorus and was prepared from wheat starch. Commercial starch contains traces of phosphorus. This can be removed readily by grinding to a fine condition and agitating with a large volume of 0.2 per cent HCl, allowing the starch to settle and decanting the solution.

The cane sugar used was free from phosphorus.

Butter fat obtained by melting butter and filtering the clear fat through paper contains small amounts of phosphorus which can be almost entirely removed by thoroughly agitating the warm fat with slightly acidulated warm water.

The fat used gave only a yellowish tint with ammonium molybdate in nitric acid solution. There was no precipitate from 5 grams after warming the test solution at 60 degrees for an hour.

In one instance where bacon fat was given to Lot I near the end of the experiment, this contained a small amount of phosphorus in unknown form.

The proteins thus purified still retained an appreciable taste, the edestin much more than the zein, probably due to the great

insolubility of the latter in water. Further constituents of the ration were corn starch, wheat starch, butter fat freed from phosphorus as described, cane sugar, milk sugar, pure glucose, cholesterine, and ash of milk. Calcium phosphate and sodium chloride were always added, and at intervals ferric chloride.

At the beginning of the experiment a ration containing 12 per cent of protein, 75 per cent carbohydrate (starch and cane sugar), 5 per cent ash of milk, 5 per cent butter fat, 2 per cent calcium phosphate, and 1 per cent sodium chloride, was made up, mixed with a small amount of finely divided cellulose from filter paper, and enough water added to make a dough. This was dried in an oven at 100 degrees C., cut into pieces and preserved in a Mason jar. The rats ate this with apparent relish for about a week, after which there was evidence of a waning appetite. The sugar content of the food was changed and they again ate more readily. At this time the food was baked thoroughly and a portion fed in this form. At one time slightly caramelized sugar was used to give a new flavor to the food. At another the food was moistened with water distilled from a strong cheese which was finely ground. This water possessed in some degree the cheese flavor and caused the rats to eat with more relish. Good results were frequently obtained by leaving fat out of the food entirely for a few days, changing it as much as possible by the methods mentioned above, then relieving the rats of these flavors by feeding the simple food mixed with fresh butter fat. This invariably induced a good consumption for a day or two. On some days the ration was presented flavored with a trace of bananna, celery, cinnamon, lemon or vanilla flavors obtained from the commercial articles. The rats generally ate the ration on such occasions but it can not be determined to what extent the consumption was induced by these substances.

As time went on it was found that when the mixed foods were not eaten readily, pure edestin would be consumed with avidity, but only for one feeding. Glucose was frequently given separately and considerable quantities were eaten. In one instance toward the end of the experiment, bacon fat, freshly rendered and filtered through paper, induced a hearty consumption when every other means failed. Cellulose, ground charcoal and bone ash were given at different times to regulate the condition of the feces. Care was also taken to change the content in the ration of sodium chloride at intervals in order to secure the change in taste which it afforded.

This ration contained no purines. Even during starvation there is a regular elimination of uric acid arising from the breaking down of cell nuclei which necessarily accompanies the functioning of the cells. The rats used in this experiment must therefore have lost nuclear material daily or they synthesized these bodies from the food supplied. Table I presents the records kept during this experiment.

TABLE I.—RECORDS OF WEIGHTS OF RATS IN LOT I WHICH RECEIVED THE INORGANIC PHOSPHORUS RATION AND NO PURINES.

Date.	Rat IV.	Rat V.	Rat VI.
	Grams.	Grams.	Grams.
May 26	170	108	124
June 2	171	104	123
" 9	172	110	125
" 16	165	100	118
" 23	173	104	118
" 30	159	101	105
July 7	150	97	105
" 14	151	99	104
" 21	154	104	111
" 28	150	100	105
Aug. 4	145	97	100
" 11	135	95	95
" 18	(77 days)	93	94
" 25		86	83
" 31		96	88
Sept. 7		90	(97 days)
		(104 days)	

Rat IV was killed Aug. 11. During 77 days it lost 35 grams in weight or 20.59 per cent of its body weight.

Rat V was killed September 7. During 104 days it lost 18 grams in weight, or 16.66 per cent of its body weight.

Rat VI was killed on August 31. During 97 days it lost 36 grams, or 29.03 per cent of its body weight.

Rat IV was in a feeble condition when killed. The other two were still apparently in good condition.

It is interesting to compare the behavior of the rats in this experiment with those of Falta and Nöggerath[32] and Jacob[33] which were fed with similar rations but with no care to induce a good consumption of food by constant change in the flavor of the ration. Their rats lost weight regularly from the beginning of the experiment. In the experiment of Jacob a rat weighing 193 grams lost on an average 11.6 grams per week and died at the end of the sixth week. Another weighing 190 lost on an average 7.7 grams per week, dying after 11 weeks. The third weighing

[32] Falta and Nöggerath, Beit. z. Chem. Physiol. u. Path., 1905, **7**: 313.
[33] Jacob, L., Ztschr. Biol., 1906, **48**: 19.

162 grams lost only 3.6 grams per week and died only after 17 weeks.

In Falta and Nöggerath's experiment in one instance a rat of 170 grams, or about two-thirds grown, was 10 grams heavier at the end of four weeks than at the beginning of the experiment. Thereafter it lost weight steadily. All of their other rats were nearly full grown animals.

It is very suggestive that in the experience of these authors those animals which were young and had not attained their growth, withstood the artificial and unpalatable ration much better than did adults. In the case of my own experiment Rat V having the smallest initial weight did better in maintaining its body weight than did the other two. In this case no decided loss of weight began until August 1, after the animal had been on the artificial ration 66 days. In all of these rats the increased palatability of the ration deferred for some time the decrease in their body weight.

Lot II of this experiment were fed the same ration as Lot I, and the same precautions were taken in both experiments to secure the consumption of food. In addition, however, they received purine bases prepared from liver, and also lean beef which was hydrolyzed with 15 per cent H_2SO_4 until the biuret reaction disappeared. It is fair to assume that the phosphorus in this product was reduced to inorganic forms. It was thought that this mixture of amino acids might be efficient in rendering the taste of the food more pleasant. It was given only in small amounts and at irregular intervals to aid in relieving the monotony of the ration.

The record of these animals is shown in Table II.

Rat VIII lost during the experiment covering 106 days, 27 grams, or 20.76 per cent of its body weight.

Rat IX lost 28 grams during the same period, or 26.41 per cent of its body weight.

It is again apparent that by adding to the palatability of the food, the time at which the steady loss of weight began was deferred to about the 50th day in the case of rat IX and to about the 60th day in the case of rat VIII.

Rats VIII and IX were still in a fairly good condition at the end of the experiment, but it was evident here as in the case of Lot I that if kept on this food the animals would die after a few weeks more. They were therefore killed for analysis.

TABLE II.—WEIGHTS OF RATS IN LOT II WHICH RECEIVED THE INORGANIC PHOSPHORUS RATION TOGETHER WITH PURINE BASES AND AMINO ACID MIXTURE FROM THE HYDROLYSIS OF BEEF MUSCLE.

Date.	Rat VIII.	Rat IX.	Date.	Rat VII.
	Grams.	Grams.		Grams.
May 23	130	106	June 12	153
May 26	122	99	June 23	168
June 2	124	104	June 30	168
June 9	130	90	July 7	164
June 16	124	104	July 14	175
June 23	135	92	July 21	180
June 30	124	93	July 28	170
July 7	118	96	Aug. 4	176
July 14	128	99		(53 days)
July 21	129	97	Aug. 11	169*
July 28	123	95	Aug. 18	163
Aug. 4	119	90	Aug. 25	158
Aug. 11	117	88	Sept. 5 died	102
Aug. 18	111	85		(32 days)
Aug. 25	103	87		
Sept. 2	100	80		
Sept. 6	103	78		
	(106 days).			

* All phosphorus left out of the ration from this time on.

Rat VII was a remarkable individual. She ate the rations offered her with unusual persistence, and as her record shows made an actual gain in body weight of 23 grams in 53 days. It was thought desirable to find how much influence the complete removal of phosphorus in any form from the ration would have on her subsequent behavior. As will be seen from the record, her decline was steady and rapid after this change.

From the experiment with Lot II it is evident that in two cases the addition of purines and of the cleavage products of meat caused no appreciable improvement in the condition of the animals. In the case of rat VII, while it did much better than any other rat in the experiment described, it is questionable whether the purines and meat cleavage products were responsible for her unusual behavior, as will appear later.

EXPERIMENTS WITH YOUNGER ANIMALS

The experience thus gained led to the belief that further experiments with still younger animals might give yet more satisfactory results. Three young white rats weighing from 35–46 grams were used in this experiment. The ration was the same as was used with Lot I and the methods of feeding were the same. Table III shows the record of these animals on this ration.

TABLE III.—WEIGHTS OF VERY YOUNG RATS ON A RATION SUPPLYING ONLY INORGANIC PHOSPHORUS AND NO PURINES.

Date.	Rat XVIII.	Rat XIX.	Date.	Rat XX.
	Grams.	Grams.		Grams.
Aug. 3	37	35	Nov. 17	46
" 11	34	31	" 24	46
" 18	42	37	Dec. 1	49
" 25	39	33	" 8	50
Sept. 2	40	35	" 15	59
" 10	42	39	" 21	54
" 17	48	40	Jan. 5	58
" 28	49	41	" 12	60
Oct. 6	51	(56 day)		(56 days)
" 13	48			
" 20	50			
" 27	54			
Nov. 3	56			
" 10	62			
" 17	60			
" 24	66			
Dec. 1	73			
" 8	76			
	(127 days)			

Rat XVIII gained in 127 days 39 grams or 105 per cent.

Rat XIX gained 6 grams in 56 days or 17.1 per cent. This rat was killed by accident, the lid of the cage falling upon it.

Rat XX gained 14 grams or 30.4 per cent of its body weight in 56 days.

CONTROL EXPERIMENT WITH CASEIN

For the purpose of comparison a control experiment was made in which a phosphorized protein was contained in the ration. Two young rats were fed the same ration at Lot I, except that in place of calcium phosphate, casein was given. The casein was prepared fresh before each feeding by precipitating separator skim milk with acetic acid, straining and washing, then dissolving the curd in ammonia and reprecipitating with acid. The casein was always mixed with the other food and was given in a perfectly fresh condition. Ash of milk was given with this ration. Casein was not given every day but usually at intervals of two or three days, the same methods being used as in former experiments to give variety to the ration. No purines, hydrolyzed meat nor commercial flavors were given since it was not desired to introduce the two first mentioned substances and a satisfactory intake of food was secured without the latter. The results are shown in Table IV. The experiment was broken off at this date, the rats being in a healthy and normal condition.

TABLE IV.—WEIGHTS OF RATS FED ON INORGANIC PHOSPHORUS RATION USED WITH LOT I AND CASEIN.

Date.	Rat XV.	Rat XVI.
	Grams.	Grams.
Sept. 28	87	83
Oct. 6	86	77
" 13	90	80
" 20	87	78
" 27	95	87
Nov. 3	93	85
" 10	103	95
" 17	108	103
" 24	108	103
Dec. 1	111	102
" 8	107	101
" 15	106 (78 days)	100

EXPERIMENT WITH NORMAL RATION

For purposes of comparison the rate of growth of normally fed rats, receiving a ration of corn, wheat and rolled oats, Table V is included.

TABLE V.—RATE OF GROWTH OF RATS FED CORN, WHEAT AND ROLLED OATS.

Date.	Rat I.	Rat II.	Rat III.
	Grams.	Grams.	Grams.
May 26	172	135	103
June 2	200	158	125
" 9	207	167	156
" 16	223	187	138
" 23	231	196	153
" 30	233	200	176
July 7	240	203	(Had young and the record was discontinued.
" 14	241	212	
" 21	245	222	
" 28	248	228	
Aug. 4			
" 11			
" 18	255	250	

EXPERIMENT WITH MATURE RAT

In order to determine whether the period in the life of the animal is an important factor in relation to maintenance with a ration such as was used in these experiments, a single full-grown rat was placed in a separate cage and given the same ration and care that was given Lot I. His attitude toward the ration is shown by the record of his weight during a period of 33 days:

DATE	WEIGHT OF RAT
Oct. 7	233 grams
Oct. 13	215 "
Oct. 25	222 "
Nov. 3	204 "
Nov. 10	195 "

The experiment was discontinued. When the animal was placed on a corn, wheat and rolled oat ration it speedily recovered its weight. It would not eat a sufficient quantity of the artificially prepared ration to supply its energy requirement.

EXPERIMENT ON PHOSPHORUS EXCRETION

In order to throw additional light on the subject of nuclein metabolism it was desired to obtain data showing the actual amount of phosphorus excreted per day when a phosphorus free ration was taken. That this is not necessarily the same as the phosphorus excreted during starvation is the opinion of Gevaerts[34] who found that in rats when fed a ration containing no phosphorus the ratio of phosphorus to nitrogen excreted fell to about one-tenth of the value during starvation. In order to obtain more data on this important question, Rat VII, whose record is shown in Table II, was placed in an inverted bell jar having a hole in the center of the dome. A screen formed a floor on which the rat could stand and move about. Asbestos, thoroughly extracted with hot nitric acid, was spread over this screen to serve as a bed. Each day the rat was removed and the bottom of the bell jar washed with a small sponge, the washings being collected in a large dish placed below. The screen floor was washed after removing the asbestos and the latter placed in the dish with the washings. Fresh dry asbestos was placed on the screen and the rat replaced in the jar. The washings and asbestos bed were then acidified strongly with nitric acid, then boiled and the whole transferred to a suction filter and the asbestos washed free from phosphorus. The washings were then concentrated, sulphuric acid added and the solution transferred to a flask and all organic matter destroyed by oxidizing with nitric acid according to the Neumann method. Phosphoric acid was determined by precipitating with ammonium molybdate in nitric acid solution and was weighed as magnesium pyrophosphate. The phosphorus excreted is shown in Table VI. This experiment was begun on August 25th and continued eight days.

The ration given during this period consisted of zein, edestin, starch, sugar and butter fat. The ash constituents given were magnesium oxide, calcium sulphate, potassium chloride, sodium carbonate, sodium chloride, and ferric chloride.

[34] Gevaerts, La Cellule, 1901, 18: 7.

TABLE VI.—PHOSPHORUS EXCRETED BY RAT VII.

Day.	Phosphorus excreted.	Day.	Phosphorus excreted.
	Grams.		Grams.
1	0.0085	7	.0012
2	.0084	8	.0051
3	.0076		
4 & 5	.0114	Total	0.0511
6	.0089	**Average**	**0.0063**

It is not possible to say how much the rat was eating during the days of this experiment, but it is known that some food was taken each day.

Gevaerts[35] found for starving rats an excretion of phosphorus in an animal weighing 210 gram, amounting to 0.007 to 0.0114 gram per day. In another experiment the amounts were 0.0086 to 0.0184 gram per day. When phosphorus free edestin and cane sugar were fed Gevaerts observed an excretion of only 0.001 to 0.004 gram of phosphorus per day for rats weighing 180 to 200 gram.

In a second trial with a rat weighing 217 grams, the writer starved it for four days, then fed edestin and cane sugar for a period of four days. The excreta were collected together for the entire period and the phosphorus amounted to 0.017 gram, or 0.0043 gram per day.

ANALYSES OF THE CARCASSES

The phosphorus was determined in the entire bodies of nine rats (exclusive of the skeletons), by boiling the rats after opening the stomach and intestines and discarding their contents and removing the skeleton, great care being taken to remove the small bones of the feet. The tissues and water in which the rat was boiled were evaporated to dryness, dried in an oven, ground and sampled. Analysis of the dry tissues of nine individuals, including all rats in Tables I and II, and three normally fed ones, calculated on the basis of the live weight less the weight of the skeleton, showed an average phosphorus content of the body of the skeleton-free living rat to be 0.19 per cent (P). This furnishes interesting data for calculation. If a rat weighing 200 grams excrete 0.005 gram phosphorus per day, since his body

[35] Gevaerts, La Cellule, 1901, **18:** 7.

would contain 0.38 gram of this element, in the course of 76 days the entire content of phosphorus in his body would change. Since of the three rats in Tables I and II which were continued beyond 100 days, the loss of body weight was 16.66, 20.76 and 26.41 per cent respectively (average 21.27 per cent), even in these experiments it would seem incontrovertible that the animals were utilizing the inorganic phosphorus supplied for nuclein and phosphatide formation. This is all the more convincing when the weights of the skeletons of these animals are taken into account. The abnormally large skeletons found in those animals receiving a large supply of inorganic phosphates is strikingly evident even though the body weight did not increase. They were not, therefore, drawing phosphorus from this source. This is in harmony with the observations of Hart, McCollum and Fuller with pigs.

Even admitting Gavaert's lowest figure: 0.001 gram per day, a 100 gram rat, containing 0.19 gram of phosphorus in its soft tissues, would metabolize 53 per cent of this during 100 days. This is not in harmony with a loss of 16 to 20 per cent of body weight in the same time.

As a possible explanation of the results here presented, it might be urged that the gains in weight observed were due to an excessive deposition of fat or an undue accumulation of fluids in the body as sometimes occurs as a pathological condition, the animals used were accordingly in most cases subjected to the following analyses: The weight of the animal at the time of death was ascertained. It was then boiled with water until thoroughly cooked, when the skeleton was completely removed from the tissues. The tissues and water in which the rat was boiled were united and the whole evaporated on the water bath to dryness. The dry tissues (less the skeleton) were then dried in an oven at 100 degrees C. for several days, carefully scraped from the dish, ground, weighed and placed in a bottle. The skeleton was dried in an oven at 100 degrees C. and weighed and afterward ignited to a nearly white ash in a muffle. The weight of the ash was obtained.

The fat in the dry tissues was extracted with ether for 24 hours, then the tissues were ground a second time and again extracted 12 hours. While this does not give the total fat of the tissues, it is believed to represent nearly all of the fat deposited as such and not the invisible fat of the organs and tissues. The tissues thus extracted were weighed and the weight taken as fat free tissue.

The data thus obtained for all the rats analyzed is given in Table VII.

TABLE VII.—COMPOSITION OF RATS IN EXPERIMENTS WITH VARIOUS RATIONS.

Ration.	Number of rat.	Weight.	Skeleton.	Dry tissues less skeleton.	Ether extract.	Ash of skeleton.	Skeleton. ÷ Weight of rat.	Fat free tissues. ÷ Weight of rat.
		Grams.	Grams.	Grams.	Grams.	Grams.	Grams.	Grams.
Normal	I	147	6.67	38.0	8.89	3.79	4.45	19.90
"	II	157	6.50	45.0	10.80	3.85	4.14	21.79
"	X	34	1.33	9.5	3.25	.68	3.91	18.39
Inorganic Phosphorus. [Later P free]	VII	102	7.14	25 5	3.42	4.49	7.00	21.76
Inorganic P.	IV	135	9.00	34.8	7.00	4.28	6.66	20.59
" "	V	96	5.78	22.0	4.01	3.02	6.02	18.74
" "	VI	88	7.50	20.5	3.63	3.18	8.5[illegible]	19.17
" "	VIII	103	6.03	21.5	2.67	3.52	5.85	17.79
" "	IX	78	4.58	14.5	.86	2.77	5.87	17.50
" "	XVIII	76	4.97	17.5	3.40	2.14	..36	18.58

The data presented in Table VII shows that while the ratio of fat free tissues to body weight varies considerably in individuals as would be expected, this variation does not point to an abnormal composition of the body of any individual examined.

The fat content and muscle and organ content of the experimental animals must be considered normal. Hence the gains in weight in the case of rats VII and XVIII are to be taken as proof of an actual increase of muscle tissue and of organ tissue. This is true also for rats XIX and XX in which a fair gain in body weight was observed.

That the composition of the organs and tissues with respect to phosphorus, calcium and moisture is always maintained normal is shown by the data furnished by Hart, McCollum and Fuller in their analysis of pigs' tissues. That the composition of the tissues with respect to other constituents is also normal can not be doubted. The per cent of phosphorus in the ash of the bones of all rats described in Table VII was found to be the same.

CONCLUSIONS

The data furnished by these experiments seem to warrant the following conclusions:

1. The palatability of the ration is a most important factor in animal nutrition. Without palatability the ration may possess all the necessary food ingredients and yet fail to nourish an animal properly.

2. The failure of previous efforts to maintain animals on a mixture of relatively pure proximate constituents of our food stuffs was due to the lack of palatability of such mixtures.

3. When sufficient care is given to changing the character and flavor of the food supplied in such simple mixtures, it is possible to induce an appreciable amount of growth.

4. Very young animals adapt themselves to a ration possessing a low degree of palatability, much better than do adults.

5. Other things being satisfactory, all the phosphorus needed by an animal, for skeleton, nuclein or phosphatide formation, can be drawn from inorganic phosphates.

6. The animal has the power to synthesize the purine bases necessary for its nuclein formation from some complexes contained in the protein molecule, and does not necessarily use purine bases of exogenous origin for this purpose.

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